Building a broodstock of the critically endangered sturgeon *Acipenser sturio*: Problems and observations associated with the adaptation of wild-caught fish to hatchery conditions

by

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ABSTRACT. - This study deals with the establishment of a confined broodstock of the critically endangered sturgeon *Acipenser sturio* L., with special emphasis on two groups of wild captive fish. One is composed of 40 juveniles hatched in the wild in 1994 and caught in the Gironde estuary in 1995. The other is made up of older juveniles (n = 8) and adults (n = 6) caught at different times. The fish were fed frozen shrimps and reared in recirculated water systems. The main aims of the study were i) to determine optimal rearing conditions, ii) to improve our knowledge of the species (sex ratio, genetics), and iii) to determine how to manage adults in captivity to produce gametes. Short trawling, rapid transportation with water from the fish habitat and maintaining them in the hatchery in water of similar salinity reduced the initial weight loss which was measured at up to 30%. Growth of juvenile fish 1994-95 was similar in fresh and brackish water till mid 2001. Weight range increased with age. Analysis of 11-ketotestosterone (11 KT) levels shows that one third of these 1994-95-fish are probably males. Of the large juveniles kept in brackish water, some required several months to resume feeding but two 10-kg fish exhibited no weight loss, suggesting a great potential for adaptation. Some breeders did not recover their initial weight for several years, with growth best described as irregularly cyclic. The large fish (10 out of 12) exhibited better growth in a 2 m deep tank compared with a 1 m tank. Out of the 14 large fish, 6 died after one to three years holding for no known reason. The 8 remaining fish are sires, 5 of which matured in 2000 and 2001. Four of the five matured in the two consecutive years. Vernalisation (11°C in winter), natural daylight, one week in fresh water in late spring to mimic upstream migration, and hormonal stimulation with either carp pituitary homogenate or GnRH analogue provided high quality semen. The genetic variability of the Gironde population, assessed from the present experimental fish, is low and all specimens share the same mitochondrial haplotype. Characterisation of mitochondrial DNA fragments suggests that the juveniles born in the wild in 1994 were produced by the same dam and sire.

RÉSUMÉ. - Création d'un stock de géniteurs d'*Acipenser sturio*, une espèce d'esturgeons en danger critique de disparition : problèmes et observations associés à l'adaptation d'individus sauvages aux conditions d'élevage.

Ce travail a pour but de contribuer à la création d'un stock confiné de géniteurs d'*Acipenser sturio* L., en particulier à partir de l'adaptation à l'élevage de 2 groupes de poissons sauvages. L'un est composé de 40 jeunes juvéniles nés dans la nature en 1994 et capturés dans l'estuaire de la Gironde en 1995. L'autre groupe est composé de 8 grands juvéniles et de 6 adultes capturés à divers moments. Les poissons élevés en circuits fermés sont nourris avec des crevettes congelées. Les principaux objectifs de travail sont :i) déterminer les conditions optimales d'élevage, ii) améliorer nos connaissances sur l'espèce (sexe ratio, génétique,…), et iii) déterminer comment on doit gérer les adultes en captivité pour obtenir des gamètes. Des traits de chalut courts, un transport rapide dans le même type d'eau dans laquelle ils ont été capturés, et le maintien dans l'écloserie dans une salinité identique à celle de l'eau où ils ont été capturés réduisent la perte de poids initial qui a pu atteindre jusqu'à 30%. La croissance des jeunes juvéniles de 1994-95 est similaire en eau douce et en eau saumâtre jusqu'au milieu 2001. L'écart des poids a augmenté avec l'âge. L'analyse des taux de 11-kétotestostérone montre qu'un tiers des animaux de 1994-95 sont probablement des mâles. Parmi les grands juvéniles maintenus en eau saumâtre, plusieurs mois ont été nécessaires à certains pour retrouver l'appétit sauf pour 2 animaux de 10 kg suggérant ainsi un grand potentiel d'adaptation à ce poids. Quelques géniteurs n'ont retrouvé leur poids initial qu'après plusieurs années, leur croissance a été irrégulièrement cyclique. Les gros animaux (10 parmi 12) ont montré une meilleure croissance dans un bassin plus profond (2 m contre 1 m). Parmi les 14 grands individus, 6 sont morts pour des raisons inconnues après un à 3 ans de stockage. Les 8 animaux restants sont des mâles, 5 d'entre eux sont parvenus à maturité en 2000 et 2001. Quatre parmi ces 5 poissons ont fourni du sperme deux années consécutives. Une vernalisation (11°C en hiver), une photopériode normale, une semaine en eau douce tard au printemps pour mimer la migration de ponte, et une stimulation hormonale avec un extrait hypophysaire de carpe ou un analogue du GnRH ont permis d'obtenir du sperme de qualité. La variabilité génétique de la population de la Gironde, estimée par les animaux objet de la présente étude, est très faible et tous les individus partagent le même haplotype mitochondrial. Une caractérisation de fragments de d'ADN mitochondrial suggère que les jeunes juvéniles de 1994 ont été produit par un seul couple de géniteurs.

Key words. - Acipenseridae - *Acipenser sturio* - Rearing condition - Growth - Feeding - Spermiation - Sex determination - Genetic variability.

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Overexploitation, damming, dredging and most probably deterioration in hydrological characteristics have led to a dramatic decrease in the European Atlantic sturgeon, *Acipenser sturio*, population since the 60's (Trouvery *et al.*, 1984). As a result, the species has been given total protection since 1982 in France and 1998 in Western Europe. As early as the late 70's, we expected to sustain the wild population artificially by restocking with fingerlings (Williot *et al.*, 1997). Up to the end of the 80's, this aim was based on the availability of wild breeders, even though they were rare and/or did not have the optimum physiological condition for reproduction. From this experience, the development of farmed broodstock was considered to be a way of providing fingerlings on a regular basis. Due to the uncertainty of the long-term maintenance of the wild population, an emphasis on conservation was added more recently. All possibilities are being investigated to increase the rate of rearing success and enhance maturation. One method is the rearing of artificially produced fingerlings (Williot *et al.*, 2000a; 2005). This is a long-running experiment, currently underway in both France and Germany (Kirschbaum *et al.*, 2000). The other method is based on acclimating small and large wild fish to farm conditions, as described in the present study.

Preliminary experiments in 1991 and 1993 were conducted according to the hypothesis that the species completes its biological cycle in fresh water, as observed in other anadromous sturgeon species *Acipenser naccarii* (Arlati *et al.*, 1988) and *Acipenser transmontanus* (Struffenegger, 1992). It was shown that direct acclimation to fresh water was possible for *A. sturio* with a total length (TL) ≥ 105 cm (~8 kg) and with a delay in food intake of about 6 months. Fish initially lost up to 20% of their body weight (Williot *et al.*, 1997). In general, nothing was known about the best conditions for cultivating the species (rearing structure, environmental condition, type of water, food,…), or how to manage the broodfish properly to allow them to produce good quality gametes. Moreover, there are no biological standards that can be used as references to assess the general health of the fish in relation to environmental conditions. As part of the establishment of an experimental broodstock, investigations on genetic variability and gender determination were performed on the confined stock in order to formulate future mating strategies to maximise genetic variability.

The main objectives of the present work were: i) to minimize the period of acclimation during which captive wild fish lose weight before resuming normal feeding, ii) to provide the fish with good rearing conditions as judged from growth rates and health status especially with regard to water salinity, iii) to assess the sex ratio of immature fish, iv) to determine the holding conditions necessary for fish to produce viable gametes, and v) to characterise the genetic variability of the fish.

MATERIAL AND METHODS

Fish

Two groups of fish are of concern. The first group is young juveniles, born in 1994 (Williot *et al.*, 1997) in the wild, and caught in 1995 in the Gironde estuary via trawl nets deployed from a Cemagref catamaran. In order to minimize damage to the fish, trawls were limited to 30 min. Forty-two fish were caught in two parts of the estuary and in different seasons (because of downstream migration of young fish): 29 fish caught during spring (March 7th-April 27th) in fresh water (upper part of the estuary), and 13 fish caught in summer (July 11th-September 8th) in brackish water (15‰) (middle part of the estuary). Fish were trucked in a water tank (2.5 x 1 x 0.8 m; L x l x h) provided with oxygen and filled with water taken from where they were caught. Upon arrival at the hatchery, fish were temporarily held in circular holding tanks (3 x 1 m; Φ x h) and weighed (mean ± sd): 170 ± 71g (n = 29) (fish from fresh water) and 528 ± 120g (n = 13) (fish from brackish water). One tank was a flow-through system supplied with fresh water at a constant temperature of 17.5°C; the other was a closed brackish water (15‰) system with mechanical and biological filters, UV treatment and oxygen enrichment column. The water temperature was equilibrated with outside air temperature. Two fish caught in fresh water died soon after arrival at the hatchery.

The second group of fish is far less homogenous than the first; it is composed of a few wild breeders (n = 6; weight range 17.5-47 kg) and some large juveniles (n = 8; weight range 4.5-10.5 kg) caught on different occasions (obtained from by-catch of commercial fisheries) and transferred to the hatchery. The sex of the latter fish was unknown upon arrival. Culture trials were initiated during late 1993 for breeders and large juveniles. The same transportation procedure as described above was used for these fish except that for logistic reasons fish were transported in hatchery water.

Upon arrival, all fish were weighed, and tagged with both pit tags and Petersen disc tags attached to the base of the dorsal fin.

Holding tanks and current water management

In the small juvenile fish rearing trials, the objective was to compare two types of water, fresh and brackish. In October 1995, two equal batches of 20 juvenile fish were placed into circular Fiberglas tanks (4 x 1 m; Φ x h). One was supplied with fresh water, and the other with brackish water (14-16‰ salinity). Fish caught in fresh water were kept in fresh water except for 7 individuals, which were transferred to brackish water in order to have two equal batches. At the beginning of the experiment, the fish weighed (mean ± sd) 234 ± 73 g and 503 ± 116g in fresh and brackish water

respectively. Each of these tanks was part of a recirculating system composed of a pump equipped with a basket to collect refused food, a mechanical filter (S = 0.63 m^2; h = 0.9 m, filled with 500 kg of sand (0.8-1.2 mm)), a biological filter (Φ = 0.76 m, h = 2 m filled with 700 l of biogrog), and a column (Φ = 0.2 m, h = 1.3 m filled with "levapack" modules with a specific surface of 144 m^2/m^3) for oxygen enrichment. These tanks were located in an isolated building which was supplied with red light from 08:00-18:00. Two fish were lost during the first few months when they jumped out of the rearing tanks; protection against accidental escape was subsequently put in place by installing vertical 0.6 m high wire fencing on the top of the tank.

Large fish were held in two Fiberglas tanks: 4 x 4 x 1 and 4 x 4 x 2 m (L x l x h) supplied with brackish water (15‰). Each tank was part of a similar recirculating system to that described above except that the biological filters were larger, with a biogrog volume of 1300 l. Flow rate was maintained at 3.5-4.5 m^3/hour. Daily renewal was equal volumes (300 to 500 l) of fresh water and seawater.

The fresh water was bacteria-free well water with constant temperature 17-18°C, de-gassed and further oxygen-enriched via a tower. Seawater was delivered by trucks and stored in large tanks with constant circulation, aeration, and UV sterilisation. Prior to this system and till late 1997, sea-salt was used to constitute artificial sea-water but due to the high cost of this solution the procedure described above is retained. Brackish water is obtained by mixing fresh water and salt water in appropriate proportions.

Temperature, salinity, pH and oxygen were checked daily with WTW™ (Germany) devices. The yearly water quality for all tanks until autumn 2000 ranged (min-max) from 16-24°C, 14-16‰, 6.8-7.5 and 6.8-7.8 mg/l. During winter 2000-2001, in order to simulate vernalisation in tanks holding large fish, water temperature was lowered to 11°C (Fig. 1B) and photoperiod was maintained similar to the natural day length. Ammonium (indophenol blue), nitrite and nitrate contents in the water were controlled monthly in the biological filter inlet and outlet with Spectrophotometer (Milton Roy, Spectronic 401) and Merck quick colour kits respectively.

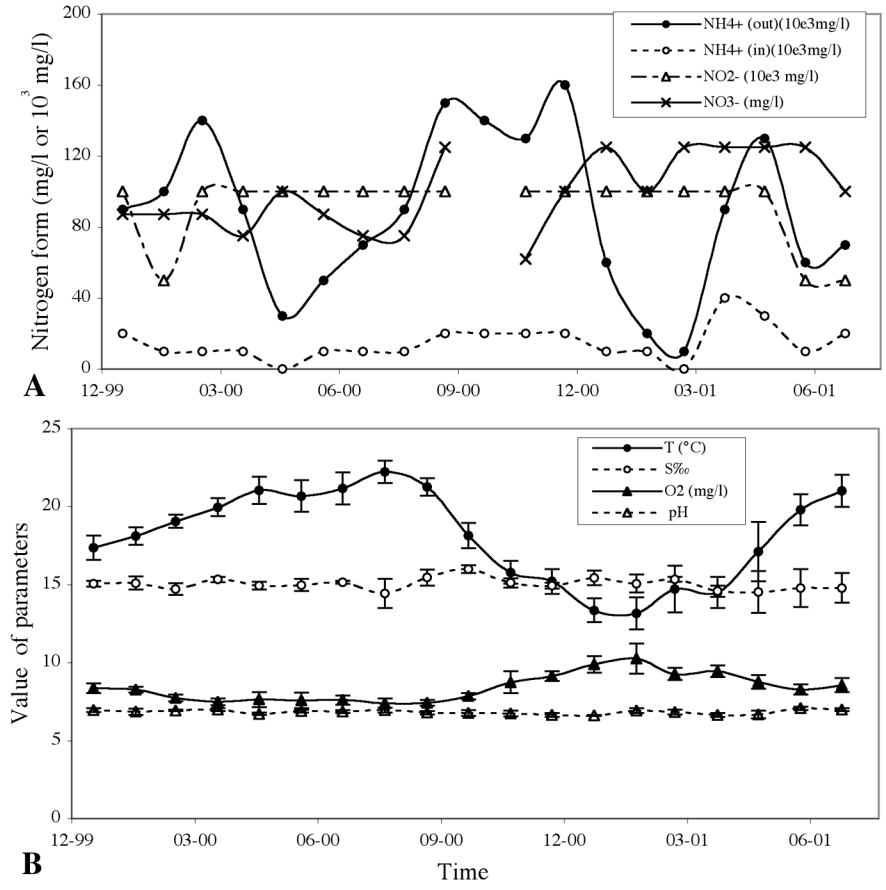

Figure 1. - Nitrogen forms of water (**A**) and physico-chemical characteristics (**B**) of a 2 m deep holding tank for large fish in 2000 and 2001. In (A), values are monthly measurements. $NH4^+$ (out) represents the ammonium content of outflow water from the holding tank (i.e. entering the biological filter), $NH4^+$ (in) represents the outflow level of ammonium from the biological filter (i.e. entering the holding tank), and in (B), values are monthly means of daily measurements. *[Formes d'azote dans l'eau (**A**) et caractéristiques physico-chimiques (**B**) d'un bassin d'élevage de 2 m de profondeur pour les gros animaux en 2000 et 2001. En (A) les valeurs sont des mesures mensuelles. $NH4^+$ (out) représente la concentration d'ammoniaque en sortie de bassin (c'est-à-dire à l'entrée du filtre biologique), NH4+ (in) représente la concentration d'ammoniaque en sortie du filtre biologique (c'est-à-dire entrant dans le bassin d'élevage). En (B) les valeurs sont les moyennes mensuelles de mesures journalières.]*

Feeding

Fish were fed frozen shrimps (tropical marine shrimps cut in small pieces, *Crangon crangon* or *Palaemonetes varians*) twice a day *ad libitum*, judged by the amount of uneaten food recovered every day from the tanks and the pump baskets. Uneaten feed was weighed in order to adapt the daily food ration, which was reduced when waste feed was > 15% of that offered. Overall, the daily shrimp portion ranged between 1.2 and 3% of tank biomass. At the beginning of these rearing trials, other types of food were investigated such as artificial food, mussels, and squid. The first

was not regularly accepted and even led to an increase in abnormalities for larvae weaned on compound diet (Williot *et al.*, 2004). The latter two food items were refused. Therefore, despite constraints (seasonal supplying, heavy costs) and risks (introducing foreign biological molecules in closed water systems), shrimp feeding has been maintained.

Fish control and sampling

Young juvenile fish were regularly weighed (every month until mid-1997 and every 3 months after this) to the nearest 1 g and 0.1 kg for small and large fish respectively. Prior to handling, fish were tranquillized by immersion for about 5 min in a bath containing essential oils of cloves added to culture water at a concentration of 40 ppm. When water temperature is low, a preliminary dilution of essential oils in ethanol (1/10; v/v) improves solubility.

Large fish, which exhibited continuous growth were biopsied for gender and sexual maturation every three months from late 1999 onwards. Gonad tissue samples were obtained and observed under stereomicroscope (ovary) or microscope (x 400 for testis). Males, which revealed an advanced maturation state were transferred to fresh water (17-18°C) for one day to 6 weeks (2000) and one week (2001) and then injected with 2 $mg.kg^{-1}$ of carp pituitary homogenate. In 2001, one of the broodfish was injected with GnRHa ([D-Phe^6] - NH_2) at a rate of 5 $\mu g.kg^{-1}$. 24-28 hours post injection, semen was collected via a dry flexible polypropylene tube carefully introduced in the genital pore and volume and motility were assessed (Williot *et al.*, 2000b). Large fish were also sampled by removing a section of the pectoral spine for age determination (Rochard and Jatteau, 1991). In younger fish, of which the sex is unknown, levels of the steroid hormone 11-ketotestosterone were used as a criterion for sex determination (Cuisset *et al.*, 1994). To perform tests, 2 ml of blood were taken from the caudal vein, transferred to heparinized microtubes and centrifuged at 8000 $r.min^{-1}$ for 8 min. Plasma was then frozen until use. The minimum detectable content of the immunoassay is 15 $pg.ml^{-1}$.

In case of unexplained mortality in any batch, another sturgeon species (Siberian sturgeon, *Acipenser baerii* B., 1869) was introduced in the concerned tank for testing the presence of a toxicant in the water.

The effect of water depth (1 m and 2 m) on growth was tested by transferring fish from one tank to another and recording weight between two successive handlings. The experiment lasted from 1998 to 2002. There were 14 transfers from 1m to 2 m tanks and 8 in the opposite direction. Twelve fish were used, some of them many times. Gain and/or loss in weight were recorded following each transfer.

Genetic analysis

Analysis of genetic variability was also carried out. Pieces of fin (around 1 cm^2) stored in alcohol were sampled either from fish reared in the Cemagref facility (CREA) or from samples taken in earlier years from wild fish (n = 20). From the fish held in CREA and subjected to present study, there were 38 large juveniles born in 1994, and 9 adult fish including the sire and the dam used in artificial reproduction in 1995 (Williot *et al.*, 2000a).

Nuclear DNA-analyses

Six different microsatellite loci (Afu-19, Afu-39, Afu-54, Afu-68: May *et al.*, 1997; Aox-23, Aox-45: King *et al.*, 2001) were investigated. Primers were labelled with 6-FAM, HEX and TET (ABI, Foster City, CA). Amplifications were performed in 25 µl containing 50 ng genomic DNA, 0.25 U *Taq* DNA polymerase (QIAGEN, Hilden, Germany), 5 pmol of each primer, 0.10 mM Tris-HCl (pH 8.8 at 25°C), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 µg/µl bovine serum albumin (BSA), 0.08% (v/v) Nonidet P40 and 100 µM of each dNTP. Amplification was performed in 30 cycles of the following steps: 30 sec at 94°C, 30 sec at 52°C up to 57°C (King *et al.*, 2001; Ludwig *et al.*, 2001), 30 sec at 72°C and a 5 min. final extension at 72°C. The size of alleles was determined using an ABI 310 DNA sequencer (ABI, Foster City, CA) with internal standards.

Mitochondrial DNA

The repeat region, including the most variable part of sturgeon mitochondrial DNA, was amplified with the primers 5'-ACCCTTAACTCCCAAAG-3' (Hetero I) and 5'-CATTTRATGGTAGATGAAAC-3' (Hetero II) (Ludwig *et al.*, 2000) under the following reaction conditions: 20 sec at 94°C, 10 sec at 50°C, and 1 min. at 72°C for 30 cycles and a final polymerization for 4 min. at 72°C. Amplifications were carried out with 1 unit Taq DNA polymerase (Oncor-Appligene), 10 pmoles of each primer, about 10 ng DNA, 100 µM of each dNTP, 2.5 mM $MgCl_2$ in 2.5 µl incubation buffer and a total volume of 25 µl. PCR products were run for sequencing analysis on a 1.5% agarose gel at 150 V for 2 h. PCR products were excised from the gel and extracted using the QIAquick Gel Extraction Kit (QIAGEN Inc., Germany). Direct sequencing was performed in both directions using an ABI 3100 sequencer (ABI, USA) following the protocol for cycle sequencing. Sequencing was performed under the following conditions: 15-40 ng DNA, 10 pmoles of each primer (Hetero I; Hetero II) and 2-4 µl BigDye RR Terminator Cycle Sequencing Kit (ABI, USA) in a Perkin-Elmer 2400 thermocycler programmed for 30 cycles of 10 sec at 94°C, 5 sec at 50°C and 4 min. at 60°C.

Statistical analyses

T-test (normality) or U-test (non normality) procedures were used to compare the effect of fresh water and brackish

water on weight gain in juvenile fish born in the wild in 1994. Normality (Kolmogorov-Smirnov test) and equality of variance had previously been checked. Significant probability level was $P < 0.05$.

χ^2 or Fisher exact test compared the occurrences of weight gain or loss following a transfer of large fish from one tank to another (1 m-tank and 2 m-tank).

Allele frequencies, allelic distribution and homozygosity values were calculated in Popgene 1.31 (Yeh and Yang, 1999). Relatedness 5.0 (Queller and Goodnight, 1989) was used for analysis of relatedness between pairs of individuals. Positive relatedness values indicate that individuals are more closely related than expected for a randomly mating population. In contrast, negative relatedness values indicate that a pair of individuals is less related than would be expected for a pair taken at random from a randomly mating population (Surridge *et al.*, 1999). Statistical significance was tested using the algorithm of Kinship 2.0 (Queller and Goodnight, 1989). Using this approach, full siblings on the one hand and parents and offspring on the other have the same degree of relatedness. Distinguishing these relationships is only possible using age data.

RESULTS

Overall management: water, feeding and survival

Physico-chemical characteristics of water

Patterns in all tanks are very similar. Changes in nitrogen forms are shown in figure 1A for the same tank as that used for physico-chemical parameters. The $NH4^+$ (out) curve representing the ammonium content of outflow water (i.e., entering the biological filter) is far higher than that of $NH4^+$ (in), which represents the ammonium content of water entering the tank (i.e., flowing out from the biological filter), thus demonstrating a good functioning of the biological filter. It has to be noted that $NH4^+$ (out) production by fish shows two minima in the reported period. The first appeared in late winter-spring 2000 as the water temperature increased (Fig. 1A). The second occurred at an earlier period in the year 2001 and corresponded to the middle period of decreasing water temperatures.

Feeding and survival

From the three types of shrimps delivered, the preferred type was *Palaemonetes varians*, a species that inhabits French Atlantic estuaries.

Within the 5-year experimental period with juvenile fish caught in 1995, only two fish died incidentally. In April 2000, 7 of the smallest fish from the two batches were removed to avoid potential food competition. Early in June 2001, three individuals from the freshwater batch died. Introduced Siberian sturgeons fed compound diet were not affected in their closed water system. Flow through water immediately stopped further mortality of *Acipenser sturio*. In August 2001, 11 specimens of the freshwater batch were lost due to human error. The experiment was therefore stopped.

Six out of the 14 large fish died on three separate occasions. One male died in April 1997 after one-year holding. Two fish, one male and the female, died in July 1995 after one- and two-year holding, respectively. The female suddenly became thinner over a two-month period (from 37 to 27 kg body-weight). In the same tank, one fish remained alive thus suggesting that deterioration of water quality was not responsible for the death of the female. The third loss concerned three fish, which died in a tank where there were three other fish, which were immediately taken out and are still alive. In this latter case, toxin is suspected as some specimens of Siberian sturgeon introduced into the tank died

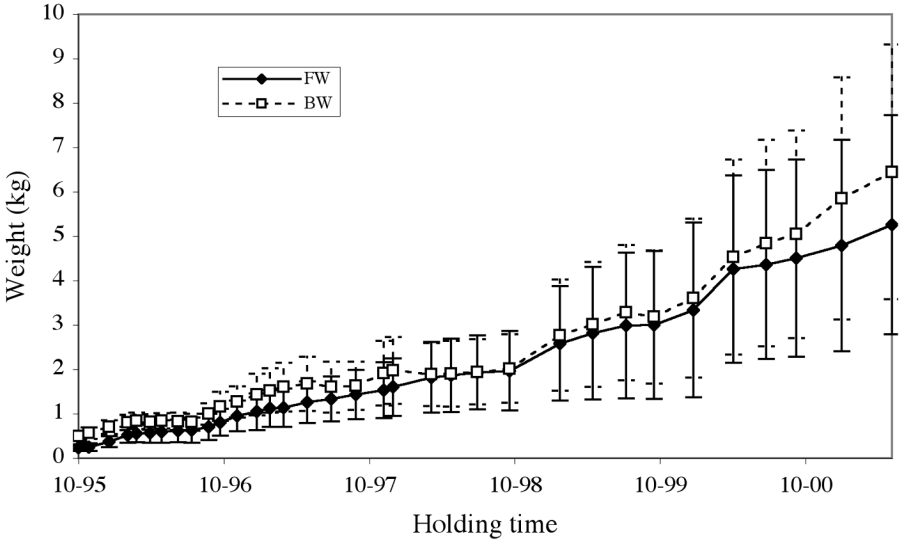

Figure 2. - Growth of juvenile *Acipenser sturio* born in the wild in 1994 and held in either fresh water (FW) or brackish water (BW) (each dot is the average weight value for 20 fish till January 1997, 19 fish till April 2000, and 15 and 16 fish after this date for FW and BW respectively). Bars are standard deviation. Though one third of distributions were not normally distributed, mean ± sd were retained. *[Croissance des juvéniles d'*Acipenser sturio *nés en rivière en 1994 et élevés soit en eau douce (FW) soit en eau saumâtre (BW) (chaque point est la moyenne des poids de 20 poissons jusqu'en janvier 1997, de 19 poissons jusqu'en avril 2000 et de 15 et 16 poissons après cette date pour l'eau douce et l'eau saumâtre respectivement). Les barres verticales sont les déviations standard. Bien que 1/3 des distributions ne soient pas normales, la moyenne et la déviation standard ont été retenues.]*

rapidly. Moreover, toxicity disappeared one week after stopping shrimp distribution.

Maximum stocking density was approximately 5 kg.m^{-2} and 0.7 ind. m^{-2}.

Growth and sex ratio in juvenile fish

The batch of fish caught during summer time resumed their initial weight faster than those caught in spring 1.2 ± 1.2 *vs* 3.6 ± 1.5 months on average. Growth curves (mean weight and sd) of juveniles raised in fresh or brackish water are similar, especially from mid-1997 onwards (Fig. 2). Prior to this date, mean weight of the brackish water batch was significantly higher than the freshwater batch. At the end of the experiment (7-year old fish) fish weighed 6.4 ± 2.9 kg and 5.3 ±2.5 kg in brackish and fresh water respectively, without any significant differences ($P < 0.23$). The range of weight variation increased with weight within the two batches. Final densities were 1 ind.m^{-2} and 5 kg.m^{-2} and 6.4 kg.m^{-2} in brackish and fresh water respectively. Growth patterns were fairly cyclical with a cycle of roughly one year, the pattern being slightly more pronounced for the brackish water batch. Among the 31 remaining specimens, only 10 exhibited 11 KT levels which suggested they were males when compared to known discriminating levels in juvenile Siberian sturgeon (Cuisset *et al.*, 1994). There were no differences in weight between these fish with regard to 11-KT level.

Growth in large juveniles and breeders

Acclimation to farming conditions

Pectoral spine analysis showed that the large fish were probably hatched in the wild between 1981 and 1989, except the largest one (the female), which was probably hatched in 1971. Most of these fish exhibited a weight loss of from 10 to 30% after arrival, with a correlation to holding time (Fig. 3). The period during which fish continued to lose weight ranged from 0 to more than 500 days. The specimen indicated by an arrow recovered and produced gametes in 2001. Weight loss vs weight upon arrival is plotted in figure 4. Group "a" fish correspond to the smallest fish (all were large juveniles), with very similar weight upon arrival and loss in the range of 10 to 40%. Those exhibiting the lowest loss were those that arrived last and benefited from all improvements in transportation processing from catch location to the hatchery. Two specimens made up group "b". They weighed around 10-kg body weight and started to feed very early, thus they did not lose any weight soon after their arrival. The third batch of fish, group "c", corresponded to breeders. Depending on the individual fish, three to five years were needed to recover arrival weight (Fig. 5). Most of the fish exhibited irregular cyclic variations in their growth (Fig. 5).

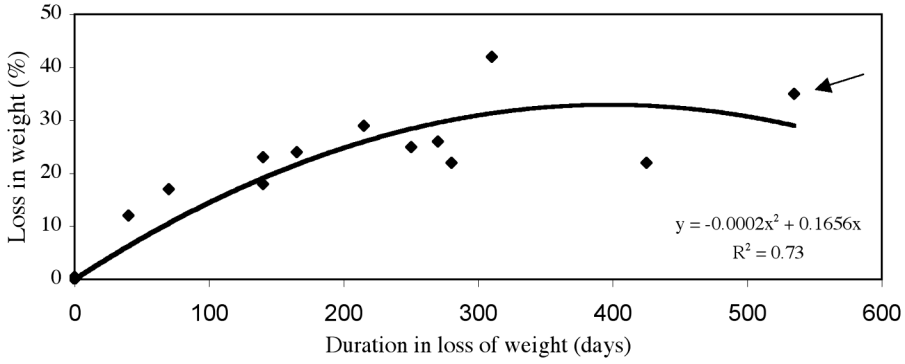

Figure 3. -Weight loss (as percentage of initial weight) of wild caught *Acipenser sturio* during the adaptation phase in the holding facility. The x-axis reports the time in days from capture to the date at which no further weight loss is observed. The arrow represents a male that provided sperm several years later. The polynomial curve is the best fit. *[Perte de poids (en pourcentage du poids initial) des* Acipenser sturio *sauvages durant leur période d'adaptation aux conditions d'élevage. L'axe des x correspond à la durée écoulée en jours depuis la capture jusqu'à la date à laquelle la perte de poids s'est arrêtée. La flèche signale un mâle qui a produit du sperme plusieurs années plus tard. Le polynôme est la meilleure approximation pour la distribution des points.]*

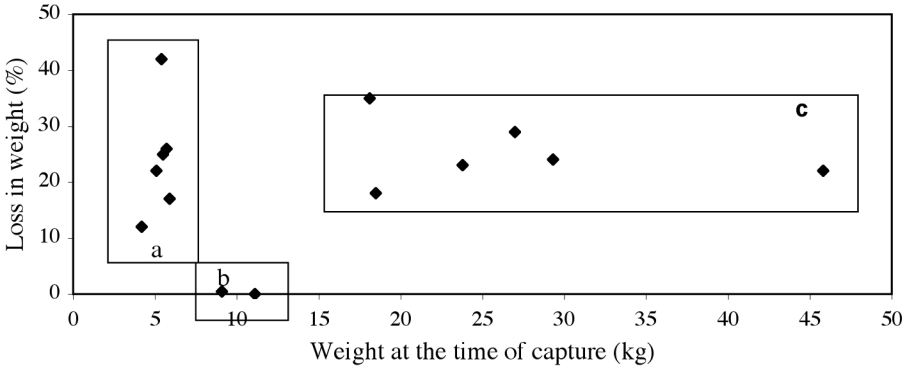

Figure 4. - Weight loss (in percent of initial weight) of wild-caught *Acipenser sturio* depending on the weight at time of capture. Group "a" fish is composed offish of very similar weight (around 5 kg) on arrival, group "b" fish is composed of two fish of around 10 kg each on arrival, and group "c" fish represents breeders. *[Perte de poids (en pourcentage du poids initial) des* Acipenser sturio *sauvages selon leur poids à leur capture. Le groupe "a" est composé d'individus ayant des poids très similaires (autour de 5 kg) à leur arrivée, le groupe "b" est composé de 2 poissons pesant autour de 10 kg chacun lors de leur capture et le groupe "c" correspond aux géniteurs.]*

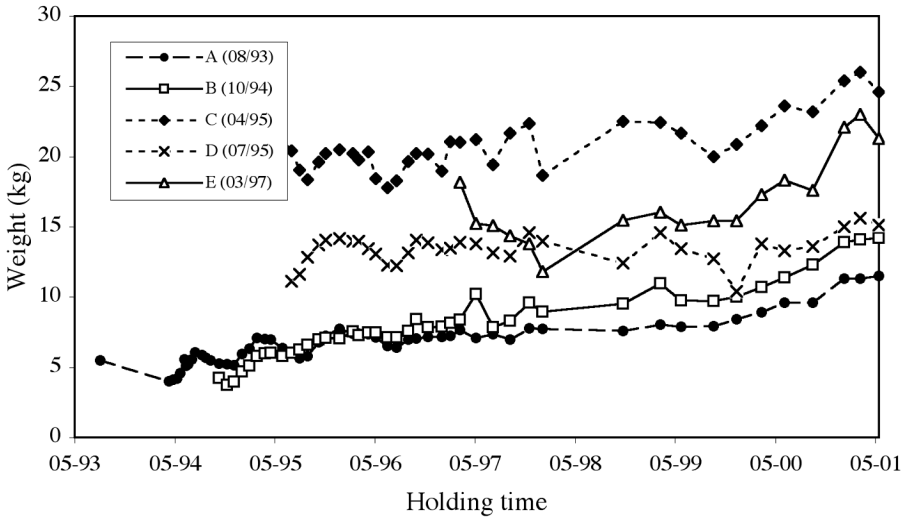

Figure 5. - Growth curves of some large *Acipenser sturio* juveniles and adults captured at different times in the wild and cultured in captivity for several years. In legend, A (08/93) means that fish A arrived in the laboratory in August 1993. *[Courbes de croissance de quelques gros juvéniles et adultes d'*Acipenser sturio *capturés à des dates diverses et mis en élevage depuis plusieurs années. Dans la légende, A (08/93) signifie que l'individu A est arrivé dans le laboratoire en août 1993.*

Influence of water depth on growth

Most weight control occurred within 3 months. Transfer of fish from a 1 m to a 2 m deep tank showed better growth than in the opposite direction (P = 0.008). Ten out of the 12 fish exhibited a better growth in deeper tank. It is worth noting that in one fish there was no weight change at transfer and in another there was weight loss at both types of transfer.

Management of mature fish

The biopsies of the 8 large fish that survived showed that they were males. Of these fish, 5 matured in early spring 2000 and 2001 (Tab. I). Four of these were mature in 2000 and 2001. Each year, two were used for experimental simulation of upstream migration. A very few spermatozoa were observed in the translucent seminal fluid in 2000. In winter 2000-2001, a pronounced vernalisation of all fish was performed. From late September 2000 to mid-March 2001, the fish grew from 14.2 ± 4.6 kg to 16.7 ± 5.5 kg and 11 ketotestosterone levels in the plasma of the fish increased from 0.5 ± 0.2 to 2.2 ± 0.9 ng.ml^{-1} but without a clear relationship between 11 KT levels and sexual maturation. In order to mimic upstream migration from brackish water (in present experiment) to fresh water, a one-week holding time in fresh water was applied before hormonal stimulation. In spite of care, tranquillisation, and the use of cannula, only 70 and 95 ml were collected from each fish in 2001 (Tab. I).

For the first time in neither sperm were there spontaneous moving spermatozoa, spermatozoa densities were fairly high, and both spermatozoa exhibited motility of 90% 30 sec post dilution in water and only 20% and 5% respectively at 1 min.

Genetic analysis

Nuclear DNA

Across all fish, each locus was heterozygous. Locus Afu-39 exhibited the highest number of alleles (seven alleles), followed by Afu-68 (six alleles) and Aox-45 (four alleles), Afu-19 (three alleles) and Afu-54 and Aox-23 (two alleles each). Interestingly, genetic variability of all loci – excluding Aox-45 – was biased by single alleles. Frequencies of dominating alleles ranged from 0.745 (allele 184 at locus Afu-54) to 0.935 (allele 124 at locus Aox-23). The most variable locus Afu-39 was also dominated by one single allele 123 (0.914). This domination seems to be influenced mainly by the very restricted number of parents involved in the natural reproduction of 1994. Overall, a very high level of relatedness was observed, ranging from 0.29 to 0.91 (value details published in Ludwig *et al.*, 2004) within the present sampled Gironde population. Moreover, significance tests regarding the existence of full siblings and/or parent-offspring relationships showed a high amount of very closely related specimens. Indeed, within the group of 9 adult alive wild-originated fish, three have significant relatedness with two of the others, three with one of the others and only one fish did not exhibited any relatedness with any other present specimen.

Mitochondrial DNA

All specimens of Gironde sturgeon had the same mito-

Table I. - Staging, preparation, and results in spermiation of confined male *Acipenser sturio*. (1) Minimum monthly water temperature in the months preceding spawning trials; (2) Number of mature and (injected) fish; (3) Spz = spermatozoa; (4) A noticeable quantity of semen was lost between genital pore walls and the external part of the tube. *[Stade de maturation, préparation, et résultats de la spermiation des mâles confinés d'*Acipenser sturio. *1) Température minimum mensuelle de l'eau dans les mois précédents les essais de reproduction ; 2) Nombre de poissons mâtures (et injectés) ; 3) Spz = spermatozoïdes ; 4) Une quantité notable de sperme a été perdue en s'écoulant entre le pore génital et les parois du tube de polypropylène.]*

Year	Minimum (1) temperature (°C)	Mature (2) (Injected)	Holding time in fresh water prior to injection	Spermiation success
2000	16	5 (2)	24 hours 6 weeks	Very few spz (3) No spz
2001	11	5 (2)	1 week	70 & 95 ml (4) semen

chondrial haplotype. The sequence was saved in EMBL (AJ249673). Using previously published data for North American Atlantic sea sturgeon (Ludwig *et al.*, 2002; Wirgin *et al.*, 2000), we observed 22 fixed substitutions between *A. sturio* and *A. oxyrinchus*. Given that the investigated part of the control region is the most variable within the mitochondrial genome, we suggest that the variability of this maternal inherited genome is practically zero for the Gironde sturgeon population. Consequently, all analysed specimens, and most probably all recent wild specimens too, share the same maternal lineage. Rare alleles (one or two) are present in only three large fish. Fish born in the wild in 1994 did not exhibit those alleles.

DISCUSSION

Acclimation

Even though most wild fish brought into captivity lost weight, we have shown that certain steps can be taken to considerably improve the adaptation of wild fish to farmed conditions. More specifically, we found that trawls of 30 min or less, rapid transportation in the same type of water in which the fish are found, and initial holding in the same water salinity, improved the survival and condition of captive wild fish.

It is interesting to note that among large fish, only the two 10-kg fish started to feed as soon as they arrived, suggesting that this size may correspond to a particular physiological state that allows the fish to adapt quickly to changing conditions.

Rearing conditions

Deeper tanks tended to improve growth. This could be a consequence of the benthic behaviour of the species of which most part of captures was signalled on the continental shelf between 20 and 30 m (Rochard *et al.*, 1997).

Salinity, 0 and half seawater, did not affect the growth of young wild-originated fish from 1.5 years old up to 7 years old. However, regarding the obtaining of motile spermatozoa from fish held the year long in brackish water (next subsection), the present findings therefore suggest that *A. sturio* might need some level of saline water to perform successful production of gametes. If so, this species most probably utilizes some hormonal control of osmoregulation (T4, T3, prolactin, etc.) and gill (Na^+-K^+)-ATPase regulation similar to that documented in anadromous salmonids (Bœuf, 1993). Investigation of this physiology should be further studied in *A. sturio* to better characterise some ecophase and rearing conditions.

In order to avoid losing potentially large quantities of fish due to different types of accident, the fish were held in different tanks. This also resulted in weakening the rearing and managing processes, as illustrated by both an unexplained mortality and a human-caused mortality. We suspect that natural food either brought a toxicant or reacted with the system in such a way that a toxin-like substance killed some specimens. This reinforces the necessity to formulate a diet which combines the advantages of both natural food (palatability, minimum detriment to fish physiology) and commercial diets (consistency of ingredients, ease of handling, absence of pathogen,…).

Even though weight recovery may take months or years, especially for captive wild fish, forming a broodstock based on wild spawners will take longer than previously expected. The present five years needed for males to mature is far shorter than the time from larvae to spawners in a natural environment, which is about 12 to 14 years minimum to our present knowledge. Growth curves with more or less regular yearly cycles for fish held at fairly constant temperature suggest that photoperiod may act on growth.

Sex ratio and maturation

The present impossibility in sex determination through use of sex steroid analysis for 21 out of the 31 suggests a low gonad growth.

It is noteworthy that a majority of males (80%) may exhibit sexual maturation over two successive years. There are no data from wild fish except one male that was caught three times at 3-year intervals (Williot *et al.*, 1997), which may suggest that recurrent spawnings are more frequent in farming conditions.

Brackish water, vernalisation, and a one-week upstream migration simulation were appropriate for male sexual maturation as, for the first time, semen was obtained from re-conditioned males of *A. sturio*. This procedure might be useful for an optimum use of males in order to cryopreserve sperm.

Genetic analysis

Genetic variation within and among individuals is important for survival and adaptation to changing environmental conditions (Frankham, 1995). Genetic variability of the *A. sturio* has decreased dramatically as a result of the decline or extinction of populations during the last centuries (Ludwig *et al.*, 2000). Conservation of the still existing genetic variability as a whole is now one of the main aims for this species. One of our goals was therefore the genetic characterisation of the last progenies born in the wild (1994) as well as of the few adult fish reared for future artificial reproduction. The number of alleles for both fish from 1994 and adult fish was reduced by ~53% compared with the genetic variability of the extinct German North Sea population (Ludwig *et al.*, 2000). The most dominating mitochondrial haplotype was also found both in archival samples from the German North Sea coast (Ludwig *et al.*, 2000) and in Gironde fish.

Overall management

It has been suggested that a minimum effective number of broodfish to be used in culture for stocking programmes should be 100 and a year-class effective population size should be at least 6, preferably with a 1:1 sex ratio for the Atlantic American sturgeon, *Acipenser oxyrinchus* (ASMFC, 1996). Even if in the future we tentatively increase the present broodstock by a few additional fish, they will be far fewer in number than the above-mentioned proposal.

In summary, acclimation, rearing, and management conditions were improved. Increased weight, spermiation of reconditioned males, preliminary data on genetic variability, and sex determination were recorded. Further investigations are needed to provide more reliable data on broodstock building with the constraints of only few high-value specimens.

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